

**A new tapeworm from the Amazon,
Amazotaenia yvetteae gen. n., sp. n., (Eucestoda: Proteocephalidea)
from the siluriform fishes *Brachyplatystoma filamentosum* and
B. vaillanti (Pimelodidae)**

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A new tapeworm from the Amazon, *Amazotaenia yvetteae* gen. n., sp. n., (Eucestoda: Proteocephalidea) from the siluriform fishes *Brachyplatystoma filamentosum* and *B. vaillanti* (Pimelodidae). - *Amazotaenia yvetteae* gen. n. and sp. n. (Proteocephalidea, Monticelliidae, Peltidocotylineae) is described from the intestine of the black-backed filhote, *Brachyplatystoma filamentosum* and from the piramutaba, *B. vaillanti* (Siluriformes: Pimelodidae) from Itacoatiara, Amazonia State, Brazil. The new genus differs from all other members of Peltidocotylineae in the morphology of the scolex, position of the vitelline follicles grouped in two elongated patches in the equatorial part of the proglottis, a smaller size of the strobila (less than 3 mm), smaller number of proglottides and smaller number of testes.

The equatorial position of vitelline follicles, their shape in two elongated patches, the uterine development in *Amazotaenia yvetteae* as well as the unusual behaviour of attachment in which individuals are grouped in compact clumps are peculiar within the Proteocephalidea. A key to the genera of the subfamily Peltidocotylineae is proposed.

The sampling and processing methodology is also described in detail.

Key-words: Eucestoda - Proteocephalidea - *Amazotaenia yvetteae* gen. n., sp. n. - *Brachyplatystoma* spp. - Brazil - Techniques.

INTRODUCTION

Proteocephalidean tapeworms are among the most common platyhelminth parasites of freshwater fishes in neotropical area. Most proteocephalid species parasitize siluriform fishes, particularly Pimelodidae (de Chambrier & Vaucher, 1999). As part of a study on the Proteocephalidea in South America, some minute tapeworms were collected from the pimelodid catfish *Brachyplatystoma filamentosum*. These new minute specimens display a series of peculiar and distinctive characters that

distinguish them from all known species and that justify the erection of a new genus. The new species is described below.

In this paper, I also give a detailed account of the preparation methods developed by Vaucher and myself since 1971 (Vaucher, 1971; de Chambrier, 1987, 1990; de Chambrier & Vaucher, 1994, 1999) and progressively adopted by other authors working on the protecephalidean cestodes (Takemoto & Pavanelli, 1996; Scholz *et al.*, 1997, Scholz & Hanzelova, 1998; Rego *et al.*, 1999).

MATERIALS AND METHODS

Sample collection

A total of 29 *Brachyplatystoma filamentosum* and 4 *B. vaillanti* were collected from the Amazon River near Itacoatiara, State of Amazonas, Brazil. The hosts were dissected and examined for parasites immediately after death. The digestive tract was dissected along its entire length and searched for cestodes under a field stereomicroscope. A few cestodes were removed immediately from the intestine, gently washed into petri dishes with distilled water, then placed into 80% alcohol or in liquid nitrogen for subsequent isoenzyme and DNA analysis. Small pieces of each of the latter specimens were isolated as vouchers for identification. Both remaining whole cestodes and voucher specimens were placed separately into vials containing a small quantity of distilled water. After 1 minute, a hot (almost boiling) 4% v/v neutral formaldehyde solution was poured directly onto the worms. In order to collect also the remaining specimens (left *in situ*), the dissected digestive tract was placed into a vial (minimum 10 times the volume of tissues) with a small amount of water. After one minute, a hot (almost boiling) 4% v/v neutral formaldehyde solution was poured directly onto the digestive tract. The worms were subsequently stored (after a minimum of 1 week) in 75% (v/v) ethanol.

Preparation of specimens

Worms were either stained in Mayer's hydrochloric carmine solution or in Weigert's Haematoxyline solution. Mayer's hydrochloric carmine as follows: 5 g Carmine Certistain (Merck) in 10 ml of a 18% HCl solution (left in contact for 1 hour) to which 200 ml of a 95% ethanol solution and a small piece of iron were added; the solution was then left to simmer for two hours and filtered when the solution was cold (modified from Langeron, 1949).

Staining with Carmine: worms were placed in Carmine for 15 minutes, rinsed with 75% ethanol, differentiated with acid 75% ethanol (HCl 0.5% in ethanol) until no carmine diffused from the worms (between 20 min and two hours), dehydrated in ethanol series (80, 95, twice 100% respectively - at least 30 minutes in each). Coiled specimens were placed carefully on a piece of Bristol board (about 25mm x 45mm) and soaked with 95% ethanol. On this surface, the worm was carefully spread, avoiding twisting, and then covered with a glass slide the same size. Bristol and glass slides will stick with one another. Specimens were placed in petri dishes with 95%,

then 100% ethanol for 30 min each. In the latter solution, the glass slide was removed carefully and the specimen was left for further 10 min. Then worms were cleared in increasing concentrations of Eugenol (clove oil) diluted in absolute ethanol (50, 75, 90 and twice 100%, at least 15 min each) and mounted as permanent preparations in Canada balsam (Fluka) (Vaucher, 1971; de Chambrier, 1987).

Staining with haematoxyline was done as follows: 5 ml 1% Haematoxyline (Fluka) in ethanol 95%, to which 5 ml of 1.2% FeCl₃ filtered solution in distilled water and 5 drops of 18% HCl were added (modified from Langeron, 1949). The worms were placed in distilled water for 5 minutes then in the staining solution for 15 minutes, destained with acid 75% ethanol, placed in tap water until a blue colour appeared (about 10 min) and then dehydrated in an ethanol series as described above for the Carmine staining.

For histological sections, pieces of strobila were embedded in paraffin wax, sectioned transversely or frontally at 13–19 µm, and after dissolution of paraffin in toluol and hydration with an ethanol series (respectively 100, 95, 75% and distilled water, 2 min each), stained in Weigert haematoxylin (10 min.), destained briefly with acid 75% ethanol, then placed in tap water until a blue colour appeared, counter-stained with 1% eosin B (Sigma) (2 min), immersed in increasing concentrations of ethanol for 2 min. each and finally in toluol before mounting in Canada balsam. Scoleces for scanning electron microscopy (SEM) were processed by following procedures: worms are deshydrated in an ethanol series (80, 95, twice 100% respectively) then transferred in amyl acetate, dried by critical point method, sputtered with gold and examined in Zeiss 940A SEM (see also Scholz *et al.*, 1998).

Type material was deposited in the Helminthological Collection of the Instituto Oswaldo Cruz (CHIOC), at the Natural History Museum (MHNG), Geneva, Switzerland and at the Natural History Museum, London (BMNH).

Measurements are in micrometers (mm) unless otherwise stated. The following abbreviations are used in the description: x = mean; n = number of measurements; CV = coefficient of variation (%).

RESULTS

Amazotaenia gen. n.

Diagnosis: Proteocephalidea, Monticelliidae, Peltidocotylineae. Very small tapeworms (less than 3 mm), with acraspedote proglottides. Unarmed scolex with four uniloculate suckers. Testes cortical, in one continuous dorsal field. Ovary medullary, with projections into dorsal cortex. Uterus cortical, ventral, with lateral and dorsal outgrowths extending into dorsal cortex. Vitelline follicles ventral, in two elongated groups in equatorial part of proglottis, occupying less than half of proglottis length. Vagina always posterior to cirrus pouch, possessing vaginal sphincter. Genital pore near anterior proglottis margin. Parasites of neotropical siluriform fishes (Pimelodidae).

Type and only species: *Amazotaenia yvetteae* sp. n.

Remarks

Amazotaenia gen. n. belongs to the subfamily Peltidocotylinae on the basis of the presence of a medullary ovary, cortical testes and vitellaria and cortical uterus with outgrowths penetrating the medulla and the dorsal cortex. Recent taxonomic works on this subfamily resulted into the erection of the genera *Jauella* Rego & Pavanelli, 1985 and *Mariauxiella* de Chambrier & Rego, 1995 (see Rego & Pavanelli, 1985; de Chambrier & Rego, 1995), the redefinition of *Peltidocotyle* Diesing, 1850 and the suppression of *Othinoscolex* Woodland, 1933 and *Woodlandiella* Freze, 1965 as its synonyms (de Chambrier & Vaucher, 1999; Zehnder & de Chambrier, 2000). Therefore, only three genera belong to this subfamily at present, i.e., *Peltidocotyle* Diesing, 1850, *Jauella* Rego & Pavanelli, 1985 and *Mariauxiella* de Chambrier & Rego, 1995.

Amazotaenia differs from the three members of the Peltidocotylinae by the morphology of the scolex which is relatively small and without metascolex. In contrast, *Peltidocotyle* and *Jauella* are characterised by the presence of metascolex, and the species of the genus *Mariauxiella* have massive scoleces. The position of the ventral vitelline follicles of *Amazotaenia* is in two groups in the equatorial part of proglottis, compared to the lateral vitelline follicles in the other three genera. In addition, the new genus can be distinguished by the smaller size of the strobila (less than 3 mm), the smaller number of proglottides (up to 7) and smaller number of testes (maximum 32) (see the diagnoses of the other three genera in the following key).

On the basis of the present results and previous studies (Rego & Pavanelli, 1985; de Chambrier & Rego, 1995; de Chambrier & Vaucher, 1999; Zehnder & de Chambrier, 2000), the following key to the genera of the subfamily Peltidocotylinae is proposed:

- 1a. Metascolex present 2
- 1b. Metascolex absent 3
- 2a. Suckers biloculate *Peltidocotyle* Diesing, 1850
Diagnosis: Proteocephalidea, Monticelliidae, Peltidocotylinae. Worm of medium size. Scolex with four biloculate suckers forming two separate cavities. Metascolex present. Ovary medullar. Uterus cortical, ventral, with diverticula occasionally protruding across longitudinal musculature into medulla. Testes in dorsal cortex. Vitelline follicles cortical, in two bands, dorsal and ventral. In South American siluroid fishes.
Type species: *Peltidocotyle rugosa* Diesing, 1850 in *Pseudoplatystoma corruscans*, *P. fasciatum*.
Other species: *Peltidocotyle lenha* (Woodland, 1933) in *Paulicea luetkeni*, *Sorubimichthys planiceps*.
- 2b. Suckers uniloculate *Jauella* Rego & Pavanelli, 1985
Diagnosis: Monticelliidae, Peltidocotylinae. Worm of medium size, wide. Scolex small, retractile. Suckers uniloculate, small. Metascolex cone-shaped, with transverse circular folds. Ovary medullar. Uterus medullar, with nume-

rous outgrowths from the diverticles to the ventral and dorsal cortex. Testes in cortex, in one dorsal field and in one or two layers. Vitelline follicles cortical, lateral, in two dorsal and ventral bands. Parasite of Neotropical siluroid fishes. Type and only species: *Jauella glandicephalus* Rego & Pavanelli, 1985 in *Paulicea luetkeni*.

- 3a. Vitelline follicles lateral. Suckers uniloculate, with powerful circular musculature *Mariauxiella* de Chambrier & Rego, 1985
 Diagnosis (modified from de Chambrier & Rego, 1995): Monticelliidae, Peltidocotylineae. Worm of medium size. Strobila acraspedote. Scolex without defined metascolex. Four uniloculate suckers with powerful circular musculature in its distal parts. Vitelline field cortical, crescentic in cross sections, sometime extending posteriorly behind the ovary. Testes cortical in continous dorsal field and in one or two layers. Ovary medullar, with two lateral lobes, each lobulate with projections into the dorsal cortex. Uterus in ventral cortex, with numerous outgrowths from the diverticles to the dorsal cortex. Parasite of Neotropical siluroid fishes.
 Type species: *Mariauxiella pimelodi* de Chambrier & Rego, 1995 in *Pimelodus ornatus*.
 Other species: *Mariauxiella piscatorum* de Chambrier & Vaucher, 1999 in *Hemisorubini platyrhynchos*.

- 3b. Vitelline follicles equatorial. Suckers uniloculate, simple . . . *Amazotaenia* gen. n.
 Diagnosis: see above.

Amazotaenia yvetteae sp. n.

Figs 1- 18

Type host: *Brachyplatystoma filamentosum* (Lichtenstein, 1819), common name: filhote da capa preta [black backed filhote].

Other host: *Brachyplatystoma vaillanti* Cuvier & Valenciennes, 1840, common name: piramutaba.

Material studied: Brazil, State of Amazonas, Itacoatiara, Rio Amazonas, holotype CHIOC 34363, 6 paratypes MHNG, 29732-29737 INVE and 2 paratypes BMNH 2000.9.4.1., all collected on 13.10.1995 by the author. Other material: MHNG, 29738-29741, 29743 INVE, 13.10.1995; 29742 INVE (*B. vaillanti*), 02.10.1995, collected by the author.

Prevalence: 1/29 = 3.4% for *B. filamentosum* and 1/4 = 25% for *B. vaillanti*.

Intensity: 66 - more than 250 specimens.

Site of infection: first twelfth of intestine, immediately posterior to the stomach. Always concentrated in small clumps of 14 to 66 individuals.

Etymology: The generic name refers to the geographic region; the specific name in honour of Yvette, the wife of the author.

Description: Monticelliidae, Peltidocotylineae. Very small cestodes, 516-2900 long and 120-475 wide, flattened dorsoventrally, forming isolated compact groups of 14-66 ($x = 32$, $n = 10$) individuals, all situated in the first twelfth of intestine, immediately posterior to stomach (Fig. 18). Strobila acraspedote, apolytic, consisting of 4-7 ($n = 29$) proglottides: 2-3 immature (up to appearance of spermatozoa in vas

deferens), 1 mature (up to appearance of eggs in uterus), 1 pregravid (up to appearance of hooks in oncospheres) and 1-2 gravid segments. Immature proglottides much wider than long (110-375 x 60-210; length/width ratio 1 : 0.40-1.00); mature proglottides slightly wider than long (200-430 x 140-400; ratio 1 : 0.48-1.60); pre-gravid proglottides wider than long to elongate (220-440 x 310-700; ratio 1 : 0.77-2.19); gravid proglottides (attached and detached) longer than wide (260-490 x 450-770; ratio 1 : 1.12-2.31) (Figs 1, 13). Tegument thick, covered with small, round microtriches on sucker margins (Fig. 14) and covered with dense filiform microtriches on anterior margin of mature proglottis (Fig. 16).

Scolex aspinose, small, slightly flattened antero-posteriorly, 230-400 ($x = 330$, $n = 29$, $CV = 14\%$) in diameter, much wider than neck (Figs 1, 15, 17). Scolex containing antero-lateral uniloculate suckers, 95-165 ($x = 130$, $n = 116$, $CV = 12\%$) in diameter. Apical organ absent.

Internal longitudinal musculature weakly developed, in particular in pregavid and gravid proglottides (Figs 11, 12), represented by fine bundles of muscular fibres (Figs 8-12). Fibres more numerous in immature and mature proglottides (Figs 8-10). Osmoregulatory canals situated at level of vitelline follicles, overlapping testes; ventral canals much wider than dorsals; at posterior extremity of each proglottis, one short, narrow secondary canal directed laterally (Fig. 2), separated from outside just by thin cytoplasmic layer of tegument.

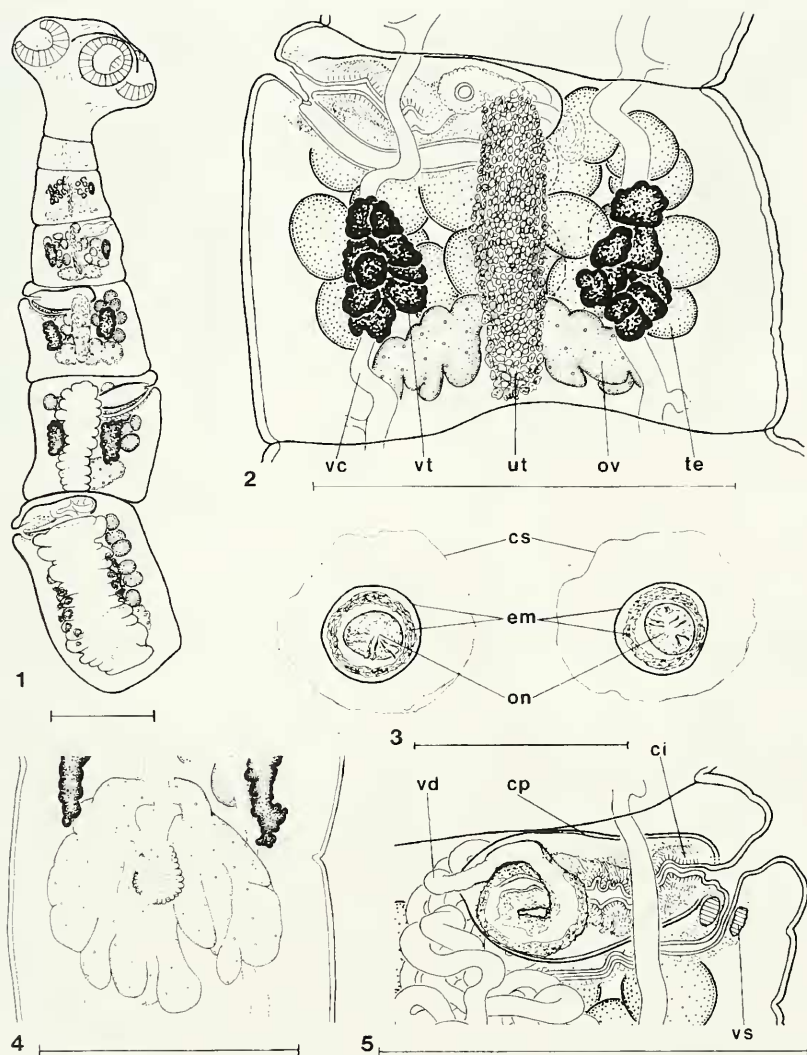
Testes cortical, numbering 19-32 ($x = 25$, $n = 48$, $CV = 14\%$), spherical to oval, 45-70 ($x = 55$, $n = 40$, $CV = 11\%$) in diameter representing 12-22% ($x = 17\%$, $n = 23$) of proglottis width, medially forming 1-2 layers, with some testes in third incomplete layer (Figs 2, 6, 7). Testes overlapping dorsal osmoregulatory canals and vitelline follicles, degenerate in gravid proglottides. Cirrus-sac pyriform to elongate (Figs 2, 5), 110-225 ($x = 160$, $n = 47$, $CV = 10\%$), basal portion extending beyond mid-line of proglottis, representing 42-64% ($x = 52$, $n = 47$, $CV = 9\%$) of proglottis width. Internal vas deferens (sperm duct) forming few loops; ejaculatory duct thick-walled; unarmed cirrus occupying more than half the length of cirrus sac. External vas deferens (sperm duct) strongly coiled, situated in anterior central part of proglottis, occupying up to 45% of proglottis width and up to 48% of proglottis length. (Figs 6, 7).

Genital atrium present. Genital ducts passing between osmoregulatory canals. Genital pore irregularly alternating, situated anteriorly at 7 to 17% ($x = 12\%$, $n = 40$; $CV = 19\%$) of proglottis length.

Vagina always posterior ($n = 76$) to cirrus-sac, thin-walled, with higher concentration of chromophilic cells at proximal end (near genital pore). Circular vaginal sphincter present (Fig. 5).

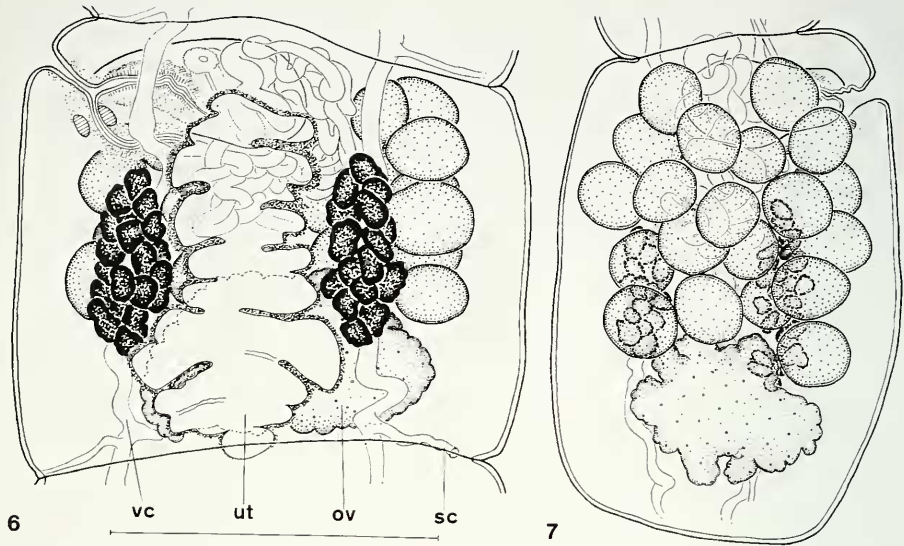
Ovary medullary, with dorsal outgrowth penetrating cortex, follicular, not clearly bilobed ventrally, occupying 42-71% ($x = 57$; $N = 39$; $CV = 12\%$) of proglottis width (Figs 2, 4). Mehlis' glands 40-55 in diameter (Fig. 4).

Vitelline follicles cortical, tightly grouped together, arranged in two elongated groups in equatorial part of proglottis, representing porally 25-48% ($x = 39$, $n = 37$; $CV = 14\%$) and aporally 23-54 % ($x = 42$, $n = 36$; $CV = 17\%$) of proglottis length, respectively, overlapping testes (Figs 2, 6-8).



FIGS 1-5

Amazotaenia yvetteae gen. n., sp. n. 1. Paratype 29733 INVE, entire worm, ventral view. 2. Holotype CHIOC 34363, premature segment, ventral view showing the disposition of vitelline follicles. 3. Eggs drawn in distilled water. 4. Holotype CHIOC 34363, detail of posterior part of a pregravid proglottis, ventral view, uterus is not figured. 5. Paratype 29734 INVE, cirrus-sac and vagina, ventral view, uterus is not figured. Abbreviations in all the figures: ci, cirrus; cp, cirrus pouch; cs, capsule; cv, vaginal canal; ; em, embryophore; lm, longitudinal internal musculature; on, oncosphere; ov, ovary; sc, secondary osmoregulatory canals; te, testes; ut, uterus; vc, ventral osmoregulatory canal; vd, vas deferens; vs, vaginal sphincter; vt, vitelline follicles. Scale bars: 1, 2, 4, 5 = 250 μ m; 3 = 50 μ m.



Figs 6-7

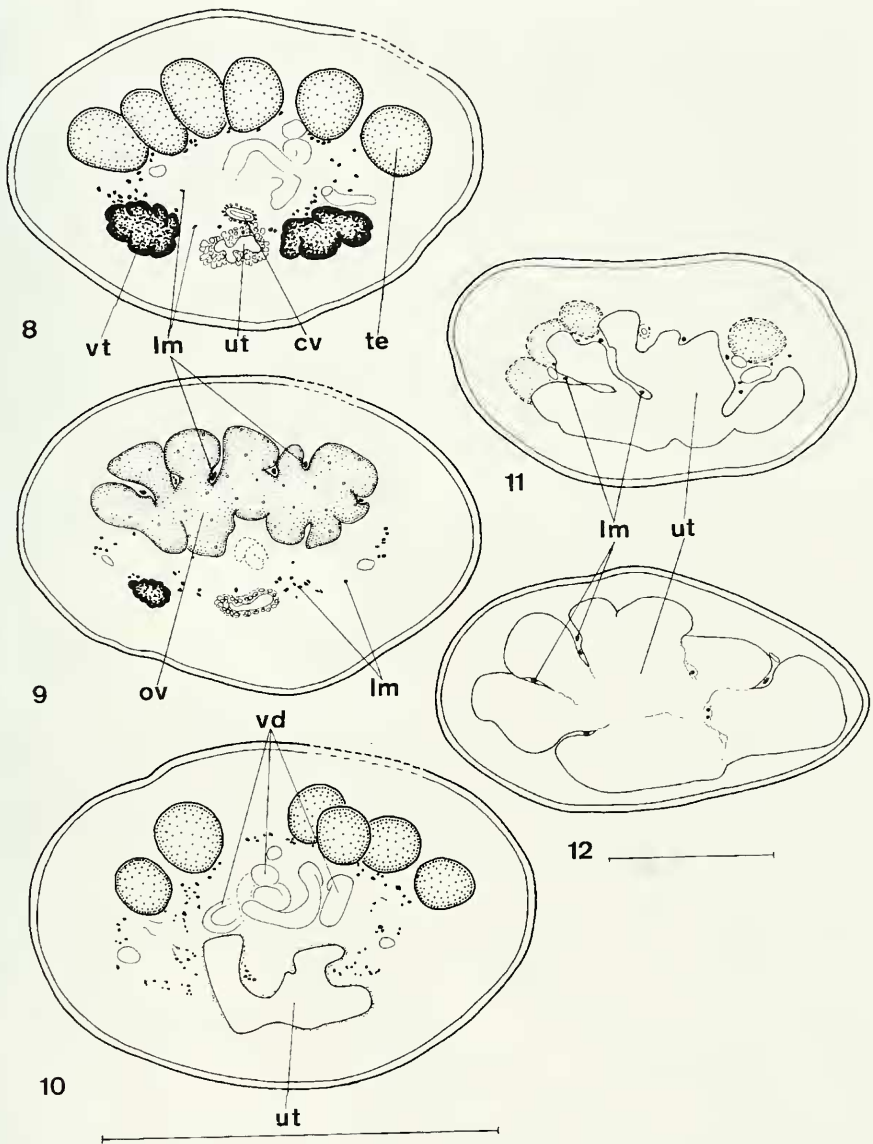
Amazotaenia yvettae gen. n., sp. n., pregravid proglottides. 6. Paratype 29732 INVE, ventral view. 7. 29742 INVE, dorsal view. Scale bar: 250 μ m.

Primordium of uterine stem cortical, already present in immature proglottides. Formation of uterus: in immature proglottides, uterine stem straight, formed by tubular concentration of chromophilic cells (Fig. 2). Irregular lumen of uterine stem present in last immature and first mature proglottides, formed by lateral and dorsal outpocketings penetrating medulla. Diverticula formed before presence of first eggs in uterine stem. In pregravid proglottides, eggs completely filling uterine stem and thick-walled diverticula (Fig. 6). In gravid proglottides, diverticula occupying up to 90% of proglottis width (Fig. 12). Pregravid and gravid proglottides with dorsal outpocketings invading medulla, reaching dorsal cortex (Figs 11, 12). Uterus with 10-18 ($n = 32$) lateral branches on each side. Terminal proglottides without uterine opening. Proglottides released into the gut before opening of uterus.

Egg shell with hyaline membrane, irregular in shape, 45-55 in diameter, with spherical, bilayered embryophore, 19-21 in diameter, thick; diameter of internal layer containing granular material 17-18; oncosphere spherical to oval, 12-14 in diameter, with 3 pairs of hooks 5-6 long ($n = 10$) (Fig. 3).

DISCUSSION

In *Amazotaenia yvettae*, the equatorial disposition of the vitelline follicles and their disposition in two elongated patches are rare within the Proteocephalidea. This disposition is observed only in *Vermaia pseudotropii* (Verma, 1928) (Gangesiinae) a



FIGS 8-12

Amazotaenia yvetteae gen. n., sp. n., 29738 INVE 8, 9. Mature proglottides, cross sections at level of vitelline follicles and at level of ovary respectively. 10. Pregravid proglottis, cross sections at level of anterior part showing the penetration of uterus in the medulla. 11, 12. Gravid proglottis, cross sections showing uterine outgrowths crossing the medulla and reaching the dorsal cortex. Scale bars: 8-10 = 250 μ m; 11, 12 = 100 μ m.

parasite of *Pseudeutropius garua* (Siluriforme) in India, but in the latter, the disposition is clearly lateral (Verma, 1928; Nybelin, 1942; Freze, 1965, Schmidt, 1986). In cross sections, the ventral position of vitelline follicles is similar to that found in another very peculiar proteocephalidean cestode, *Vaucheriella bicheti* de Chambrier, 1987 (Zygobothriinae) parasite of *Tropidophis* cf. *taczanowskyi* (Serpentes) in Ecuador, but the latter shows vitelline follicles in a posterior position (de Chambrier, 1987).

The minute size of *Amazotaenia yvettae* (500-2900 µm) is also remarkable and can be compared only with *Proteocephalus microscopicus* Woodland 1935, a parasite of *Cichla* spp. (Pisces: Cichlidae), which is 1540-2020 µm long according to Woodland (1935c) or 2050 to 2780 µm long according to Takemoto & Pavanelli (1996). The minute size is also linked with a very small number of proglottides in both species: 4-7 for *Amazotaenia yvettae* and 6-14 long according to Woodland (1935c) or 6-12 according to Takemoto & Pavanelli (1996) for *P. microscopicus*.

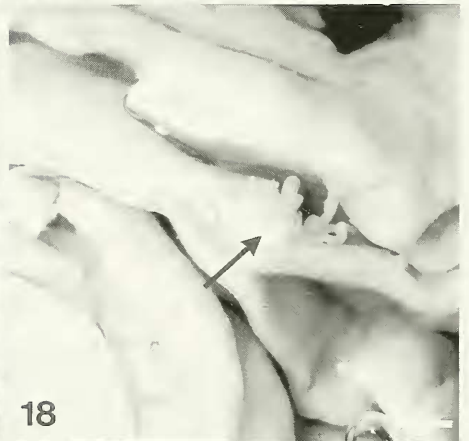
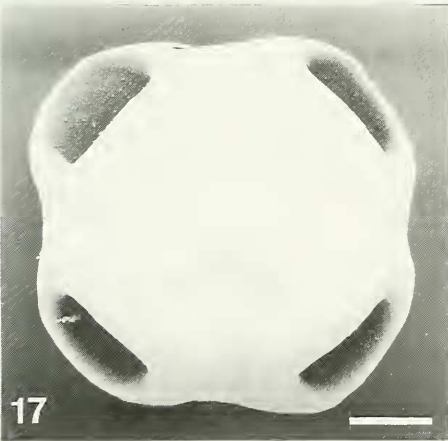
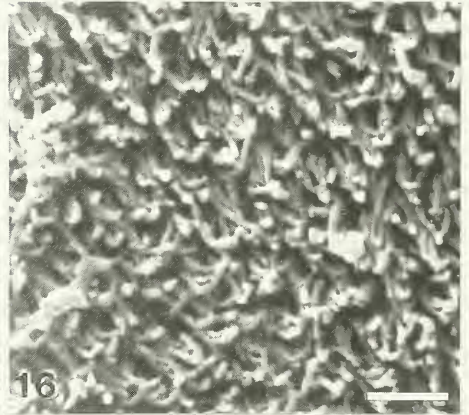
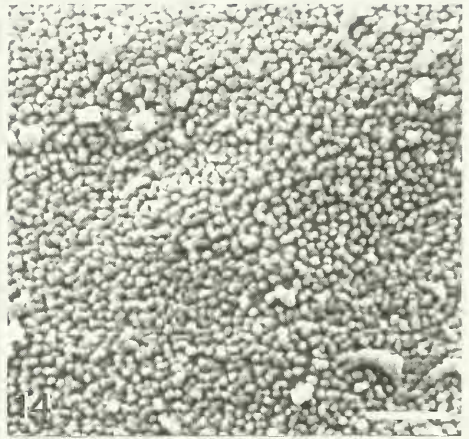
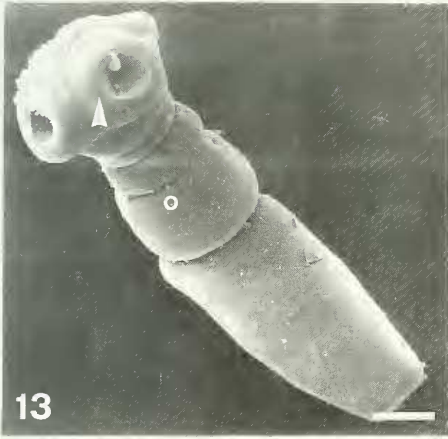
Amazotaenia yvettae is also characteristic by its unusual behaviour of attachment in which individuals are grouped in isolated compact clumps of 14 to 66 specimens. This behaviour is, to my knowledge, unique to the Proteocephalidea. They are all situated in most anterior part of the intestine (first twelfth) and are deeply embedded, perforating the epithelium into the lamina propria, generating a tissue reaction. Isolate attached specimens were never found. The specimens collected from *B. vaillanti* were identical to those from the type host and had the same site. Only one clump was found in *B. vaillanti*.

The type-host is parasitized by three other proteocephalidean species which occur in particular sites in the gut (see de Chambrier & Vaucher, 1997): *Amphoteromorphus piraeeba* Woodland, 1934 occurs from the second twelfth to the five twelfth of the intestine; *Endorchis piraeeba* Woodland, 1934 occurs from the five twelfth to the eight twelfth of the intestine; *Nomimoscolex piraeeba* Woodland, 1934 lives in the six twelfth to the ten twelfth of the intestine. As *Amazotaenia yvettae* occurred in the first twelfth, it did not share its site with any other co-parasite. *E. piraeeba* shared a part of its habitat with *A. piraeeba* and *N. piraeeba*, but the two latter species occurred in distinct sites.

The uterine development is unusual among the Proteocephalidea and is similar to that of *Mariauxiella* (de Chambrier & Rego, 1995). The uterus possesses not only lateral branches, but also has outgrowths, fan-like in cross sections, crossing the medulla and reaching the dorsal cortex. Furthermore, another peculiarity is shared with *Mariauxiella*: the medullary ovary in *Amazotaenia yvettae* also possesses obvious outgrowths into the dorsal cortex. With regard to the classification of Woodland

FIGS 13-18

Amazotaenia yvettae gen. n., sp. n. 13-17. Scanning electron micrographs. 13. Entire worm, (arrow and white circle indicate regions of tegument illustrated in Figures 14 and 16, respectively). 14. Enlarged view of margin of sucker. 15. Scolex, lateral view. 16. Enlarged view of anterior margin of mature proglottis. 17. Scolex, apical view. 18. Detail of the anterior part of the gut showing a compact clump of the new worms. SEM photos by Dr. J. Wüest (Geneva). Scale-bars: 13 = 100 µm, 15, 17 = 50 µm, 14, 16 = 1 µm, 18 = 1000 µm.



(1933 a,b,c, 1934 a,b,c, 1935 a,b,c), we suggested (de Chambrier & Rego, 1995, p. 63) that *Mariauxiella* could be placed in a new subfamily. *Amazotaenia* shares with *Mariauxiella* these characters which could justify the placement of both genera in a new subfamily. Recent studies on proteocephalidean systematics with molecular techniques (Zehnder & Mariaux, 1999; Zehnder & de Chambrier, 2000; Zehnder *et al.*, 2000) demonstrated the paraphyly of the two Proteocephalid families, the Proteocephalidae and the Monticelliidae, and suggested the polyphyly of the genera *Proteocephalus*, *Nomimoscolex* and *Ophiotaenia*. In this respect, it is not appropriate now to create a new subfamily in order to accommodate those taxa, i.e. *Amazotaenia* and *Mariauxiella*. Molecular approaches integrating detailed morphological studies could be decisive for improving our understanding of the systematic structure of the Proteocephalidea.

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